MELAMPSORA LINI (PERS.) LÉV. UREDOSPORE LONGEVITY AND GERMINATION.

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(Three Text-figures.)

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Synopsis.

During genetical investigations of rust resistance at Sydney University between 1948 and 1953 it was found that a boiled aqueous extract of host tissue greatly stimulated the germination of stored uredospores of *Melampsora lini* (Pers.) Lév. (Kerr, 1952). Exploratory studies were carried out to determine the relative efficiency of water, gelatin solution and aqueous host extract as germinating media for these spores. These showed that germination of stored spores on water was highly capricious, that better but still somewhat capricious germination obtained on gelatin, while maximum germination was obtained on the host extract. Preliminary experiments were carried out to determine the nature of the substance or substances responsible for the germination stimulating potential of the extract. These showed that the substance was part of or comprised the ether-soluble oily fraction adsorbed by activated charcoal.

Other experiments were set up to determine the longevity of uredospores of different races of the pathogen under different storage conditions. These showed a significant difference between races when temperature and specially humidity were carefully controlled. Low temperature and intermediate relative humidity were most favourable to the maintenance of uredospore viability during storage.

Introduction.

In any experiment depending on infection of host material or in which the results are assessed in terms of the germination of fungal spores it is essential to obtain maximum germination of the spores. Since water is the most common natural germinating medium it is one of the most widely used in the laboratory (Hwang, 1942; Prasada, 1948). Many workers have found that water was a poor germinating medium even for fresh spores with no dormant period (Noble, 1924; Wilcoxon and McCallan, 1943). Germination was often improved on a decoction of host tissue (Chiu and Walker, 1949). Increases in germination often as good as that with host tissue were sometimes induced by extracts of non-host plants. Germination was also improved, in some cases, by the addition of chemicals to the germinating medium (Thiele and Weiss, 1920; Noble, 1924). The favourable effect of the host tissue was not always confined to an improvement in germination. Chupp (1917) found that spores which failed to germinate at room temperature did so at this temperature when young seedlings were added. Chiu and Walker found that the optimal temperature range for germination of cucurbit black rot spores on water was 20°C. to 24°C., but was 24°C. to 28°C. on plant extract. Whitehead found that spores of *Urocystis cepulae* germinated slowly on water, but much faster on onion juice.

Sharvelle (1936) found that the percentage germination, the length of the germ tubes, and type of germ tubes of *Melampsora lini* uredospores sown on cold extracts of different varieties of flax and linseed were correlated with the rust reaction of the variety to the race used. The effect was destroyed by brief heating of the extract. Cold extracts of immune and resistant varieties retarded the percentage germination and length of the tubes, but extracts of susceptible varieties neither retarded nor stimulated germination.

It is generally agreed that low temperatures and low humidities are most favourable for the maintenance of uredospore viability. Bailey (1923) reported an optimal humidity range of 20% to 40% RH for uredospores of *Puccinia helianthi*. Raeder and Bever (1931) studied the effects of five humidities (100%, 76%, 49%, 25% and 1·5% RH) on the longevity of *P. glumarum* uredospores. They survived best at 49% RH at most temperatures but in the range −2°C. to 5°C. they survived longest at 75% RH. Chester (1946), summarizing results of many workers, showed that uredo-

spores of *P. triticina* remained viable for over 1,000 days at 5°C. and for at least 100 days at temperatures of 0°C. to 20°C.

Longevity seemed to be affected by such factors as the time of year when the spores were produced (Beauverie, 1924), the rust reaction of the variety from which the spores were collected, and the degree of maturity of the spores (Chester, pp. 116-117).

Lyophilization is probably the most successful method of storing spores. It is widely used for bacteria and fungi (Raper and Alexander, 1945) and has been successfully applied to the storage of uredospores by Sharp and Smith (1952). After 500 and 300 days respectively uredospores of *P. coronata* and *P. gr. avenae* kept in a vacuum at low temperature were scarcely less viable and no less infective than they were soon after storage.

There has been no detailed study of longevity of uredospores of *Melampsora lini*. Hart (1926) found that they remained viable for seven weeks at 7°C. and 60% RH. Flor (1935) reported that they remained viable for two to four months at 4°C. and for more than a year if kept in tightly stoppered tubes at −5°C. (1945). Waterhouse and Watson (1943) observed that race A spores seemed to remain viable longer in cold storage than spores of other races. Prasada (1948) concluded that spores stored best at 5°C. to 7°C. but became inviable after 22 weeks, and appeared to remain viable slightly longer at 10°C. to 15°C. than at 0°C.

Experimental Methods.

Uredospores used in these studies were taken from samples stored in a refrigerator as reserves for the genetical investigations. They were collected from fully susceptible varieties grown in glasshouses, with the exception of a few samples collected from plants growing in a fluorescent light room. Spores were collected by dusting them from pot-grown plants onto glazed paper, and stored in small glass phials 1½″ to 2″ long and about ¼″ diameter. They rarely occupied more than 25% of the total tube volume, usually 10% or less. The tubes were stopped with labelled corks, and stored within an hour or two of collection. The tubes were kept in a brass tray which minimized slight daily fluctuations in temperature caused by periodic opening of the door. The samples were removed as a group to room temperature about four times a year during defrosting. The temperature otherwise remained fairly constant at 0°C. to 2°C.

Samples selected for study were removed to room temperature and allowed to adjust to this temperature for at least half an hour. Spores were shaken from the tubes onto the surface of the germinating media, and dispersed evenly by vigorous whisking with a looped piece of flexible wire. Whisking was continued until checks under the microscope showed that all clumps of spores had been broken up. The spores were germinated in closed petri dishes kept in the dark at a constant temperature of 15°C. Percentage germination was measured (usually after 40 hours) by counts of surface spores under the high power of the microscope from successive fields across the diameter of the petri dish.

The linseed extract was prepared by bringing distilled water to the boil, adding fresh seedling or young plant tips at the rate of one gramme green weight to 25 c.c. of water, and boiling for twenty minutes. The solution was decanted off after removing any surface scum and used without filtering or further treatment.

In the early studies, due to the pressure of the genetical and race survey work, it was not always possible to make actual counts. But, whenever possible and unless otherwise stated, germination on extract and gelatin was determined from counts of 600 to 700 spores. Spores sown on water were always too mobile to count. When it became necessary to make estimates care was taken to overestimate rather than underestimate percentage germination on water, to guard against any bias in assessing the germination stimulating potential of the linseed extract.

Experimental Results.

Germination on Water, Gelatin and Host Tissue Extract.

Spores sown on linseed extract usually germinated much better than they did on gelatin or water. It was assumed that maximum germination was obtained on the

extract. Failure of spores to realize maximum germination on the other media was attributed to storage hardening. This term is used to denote that conditioning of the spore wall or spore contents by which it becomes increasingly difficult for spores to germinate to the full extent of their viability. Samples tested during this project sometimes became severely storage-hardened in less than a month. This was exceptional. Most accessions were not noticeably storage-hardened until they had been stored for more than a month.

TABLE 1.
Germination of Uredospores Stored for One Month.

Race.	Storage.		Percentage Germination.	
	Temperature. °C.	Humidity. RH Percentage.	Water.	Extract.
2	0	25	40	97
1	0	50	25	97
2	0	75	20	91
2	3·5	25	5	98
1	3·5	75	0	52
6	10	75	5	15
Average	.. .. ..	.. ..	15	75

Samples of spores of three races, randomly selected from a range of different storage conditions and tested on water and extract, showed that spores become storage-hardened under most if not all conditions of storage. Spores stored for a month were 300% more germinable on extract than they were on water. Storage-hardening was still more pronounced after four months' storage.

TABLE 2.
Germination of Uredospores Stored for Four Months.

Race.	Storage.		Percentage Germination.	
	Temperature. °C.	Humidity. RH Percentage.	Water.	Extract.
1	0	25	3	71
6	0	25	5	83
2	0	25	0·5	74
1	0	50	15	82
6	0	50	10	95
2	0	50	3	93
6	3·5	50	5	94
6	10	25	0·2	41
1	10	50	0·1	38
Average	.. .. ..	.. ..	5	75

Spores stored for longer periods rarely germinated well on water, though some samples gave up to 60% germination after more than four months' storage. There was no consistent correlation between the degree of storage hardening and the period of storage.

Gelatin was used to counter the mobility of the spores on water, to obtain an accurate measure of spore germination on an inert medium. Experiments were carried out to determine the most satisfactory concentration for germination. One-eighth per cent gelatin solution gave the most satisfactory results over the range 0°C. to 21°C., but there was little difference in the efficiency of concentrations from one-quarter per cent to one-half per cent at the optimal temperature of 15°C. Spores gave a high

percentage germination on one-half per cent gelatin, but the tubes were stunted. At higher concentrations germination was inhibited.

Cold plant extracts were prepared by macerating host tissue in solution in a Waring blendor. This stimulated germination of some stored samples, but most samples failed to germinate any better than they did on water. The same extract boiled stimulated most of the stored samples. Four per cent linseed extracts gave the highest germinations. Concentrations as low as 1% induced quite good germination. Below this the solution lacked the surface properties of the standard extract, the spores were

TABLE 3.
Effect of Concentration of Gelatin on Germination.
Percentage Germination of Freshly Collected Race 1 Uredospores.
Concentration of Gelatin.

Temperature.	1/16 %	1/8%	1/4%	1/2%	1%	2%
5° C.	90	98	98	80*	15*	1*
15° C.	89	93	91	91*	9*	3*
21° C.	65	86	74	80*	4*	1*

* Stunted germ tubes.

too mobile to permit accurate counts, and the percentage germination fell away. Extracts prepared from immune and susceptible varieties, from typical flax and typical linseed varieties were equally effective in stimulating germination.

Comparative studies were made on the three media at optimal temperatures. Results in Table 4 were typical of those given by most if not all races stored for more than five months.

Germination was always poorest on water. The spores were very mobile on the surface of the water. Germination was negatively hydrotropic. Most of the tubes were long, smooth, hyaline and almost unbranched. After several days they formed an anastomosing network of apparently fused tubes. Most samples germinated better on gelatin than they did on water. The increase ranged from a very slight increase

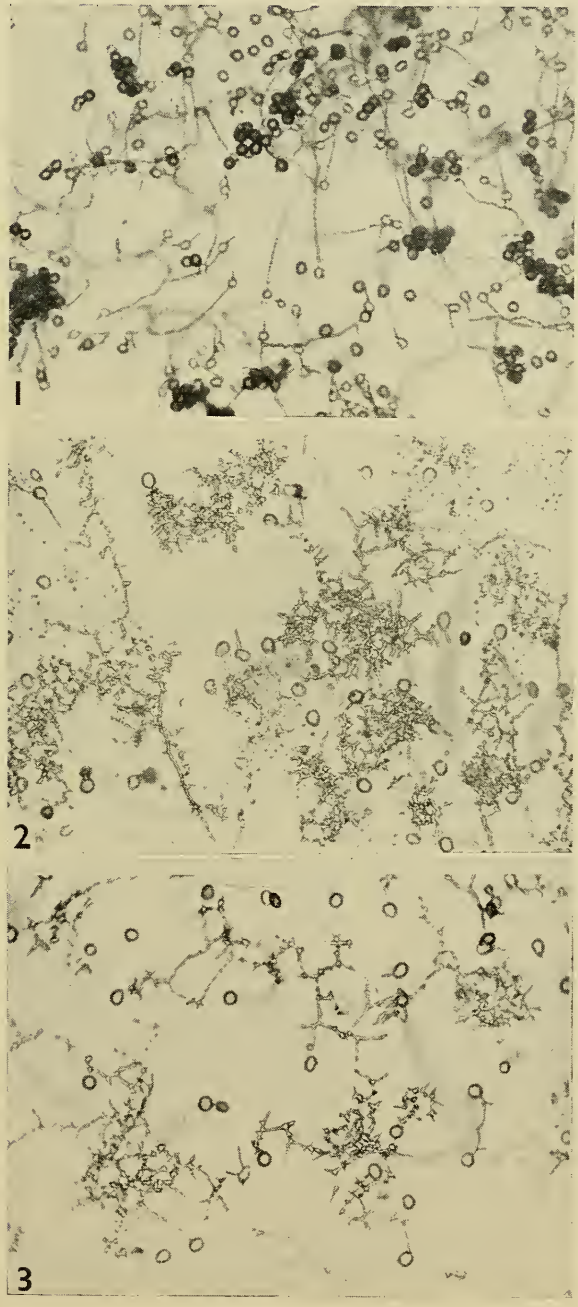
TABLE 4.
Comparison of Percentage Germination on Gelatin, Linseed Extract and Water.

Race.	S.U. Accession Number.	Date Collected.	Months Stored.	Percentage Germination on Media Listed.		
				Linseed Extract.	Gelatin 1/8%.	Water.
2	492	2: 6:52	11	18	0·2	0
5	519	22:11:52	5½	75	19	2
2	548	22:11:52	5½	95	62	10
8	555	16:11:51	18	26	15	5
3	556	16:11:51	18	25	13	5
1	557	25:10:52	6	91	3	3
8	564	18:11:52	5½	94	55	10
New Zealand Race 3		22:11:52	5½	78	55	15
		Average ..	.. ..	63	28	6

to an increase almost as great as but never equal to that induced by the extract. Germination was relatively negatively hydrotropic, but many tubes grew along and some below the surface. The tubes like those on water were long, hyaline and almost unbranched (Text-fig. 1).

The spores germinated faster on the extract than they did on the other two media. Some tubes grew aerially and were typically unbranched. Most tubes grew along or just below the surface. After reaching a length of two to three spore diameters they branched profusely distally to produce a network best described as stag-horn (Text-fig. 2). This type of branching was most pronounced among the Punjab-attacking (PA)

races, and somewhat less pronounced with some non-Punjab-attacking (NPA) races (Text-fig. 3).



Text-figures 1-3.

Maximum germination was obtained on each medium at 13°C. and 15°C. Experiments were set up to determine the effects of higher temperatures on germination on the different media.

In an early experiment samples of race 2 were tested on extract and water at 13°C. and 23°C. (Table 5). There was an appreciable reduction of germination on both media at the higher temperature. The adverse effects of the higher temperature were most pronounced on water.

The greater the storage-hardening of the spores and/or the more adverse the conditions for germination, the greater the discrepancy between the observed germination and the known viability assessed from the germination at 15°C. on extract (optimal germination conditions). The experiment summarized in Table 6 showed clearly that germination of the uredospores was conditioned by the temperature tolerance of the race, the temperature of germination, the nature of the germinating medium and the storage-hardening of the spores.

TABLE 5.

Estimated Percentage Germination of Race 2 (Acc. 492) Uredospores on Water and Extract at 13° C. and 23° C.

Medium.	Temperature. °C.	Period Stored at 0° C. in Months.												Average.
		1½	3½	4	4	4	4	4	4	5	5	5	5½	6½	
Extract ..	13	90	95	90	90	90	90	90	90	95	95	95	85	85	90
Water ..	13	10	20	15	10	30	10	60	80	60	10	50	10	50	32
Extract ..	23*	84	84	50	41	40	90	73	88	80	71	84	66	73	71
Water ..	23	4	½	0	0	0	1	½	1	1	½	0	1	0	1

* Count of 500 spores.

Each sample gave maximum germination on the extract at 15°C. The percentage germination under these conditions was taken to be the viability of the spores. Freshly collected spores of any race germinated to the full extent of their viability at 15°C. and 20°C. on extract. The slightly reduced germination at 20°C. on extract of races 1, 5 and 6 stored for two months, and of the short-stored race 11, indicated a slight storage-hardening of these samples. The other two short-stored races 5 and 13 (stored for only one week) germinated equally well at both temperatures on extract and were presumably not storage-hardened. (The extreme storage-hardening of this sample of race 11 after only two weeks' storage is unusual but not without parallel. Its storage-hardening was fully confirmed by its very poor germination on gelatin.)

TABLE 6.

Germination of a High-Temperature-Tolerant Race and Four Other Races on Three Germinating Media at 15°, 20° and 25° C.

		Punjab	Non-Punjab-Attacking Races.				
Race		1	5	6	5	13	11
S.U. Accession number ..		507	496	528	519	582	568
Period stored		2 months	2 months	2 months	1 week	1 week	2 weeks
Medium.	Temperature.						
Extract ..	15° C.	85	92	94	99	97	89
Gelatin ⅛% ..	24 hours.	66	63	62	47	58	12
Water ..		65	50	50	20	10	5
Extract ..	15° C.	86	92	93	—	—	—
Gelatin ..	48 hours.	66	65	70	43	58	9
Extract ..	20° C.	78	88	86	98	97	79
Gelatin ⅛% ..	40 hours.	60	47	34	40	58	10
Water ..		40	30	15	2	—	5
Extract ..	25° C.	70	10*	13*	0	30*	3*
Gelatin ⅛% ..	40 hours.	2	0	0	0	0	0
Water ..		1	0	0	0	0	0

* Germ tubes abnormal.

Very poor germination on gelatin was a reliable indication of marked storage-hardening when the spores were highly germinable on extract. But percentage germination on gelatin was no accurate indication of the measure of storage-hardening. This was clearly indicated by the fact that non-storage-hardened races germinated more poorly on gelatin at 15°C. in this experiment than spores of the races stored for two months. Despite this, results on gelatin gave quite good indication of the temperature effects on germination. Since the short-stored races 5 and 13 were not storage-hardened and were virtually freshly collected a marked reduction in percentage germination at the higher temperature was not expected. Germination of these races on gelatin at 15°C. and 20°C. confirmed this. The two long-stored races 5 and 6 germinated much more poorly at the higher temperature. This agreed with the general observation that 20°C. has an adverse effect on the germination of storage-hardened spores. Race 1, however, owing to its high-temperature tolerance, germinated almost as well at 20°C. on gelatin as it had at 15°C.

TABLE 7.

Effect of Temperature on Germination of Stored Spores Sown on Extract.

Temp.	Race ..	1	1	1	1	1	17	13	18	7	15	17
	Accession	613	613	507	621	624	604	582	607	550	599	604
	Months stored ..	9	1¼	¾	½	1¼	½	1	½	½	½	11
15° C.	.. ..	90	97	100	100	98	96	93	89	83	95	74
20° C.	.. ..	90	97	100	99	99	95	91	61	80	96	65
25° C.	.. ..	87	92	98	99	96	87	93	5	70	97	4

The adverse effect of high temperature was most pronounced at 25°C. None of the NPA races germinated normally on any of the media. Some gave a slight percentage germination on extract, but germ tube development was quite abnormal. Germination was entirely suppressed on gelatin and water. Race 1, however, germinated well on the extract. But almost complete suppression of germination on water and gelatin suggested that the temperature was beyond the optimal.

An experiment summarized in Table 7 confirmed the conclusions drawn from the preceding experiment. Five different accessions of Punjab-attacking race 1 and six NPA accessions, including five different races, were tested on extract at 15°C., 20°C. and 25°C. The spores held at 25°C. were pre-exposed to optimal germinating temperatures for twenty minutes immediately after sowing before being placed at 25°C. Percentage germination was checked after forty hours and the type of germ tube development noted.

Each race 1 accession gave excellent percentage germinations at each temperature with only a slight falling off at the higher temperatures. Two of the six NPA accessions were appreciably less germinable at 20°C. than they were at 15°C., and gave extremely poor germination at 25°C. Both were extremely storage-hardened, though one had been stored for only two weeks. Tests on gelatin fully confirmed their storage-hardening. The other four NPA accessions germinated well at the higher temperatures and were apparently not appreciably storage-hardened. The high germination of these accessions at 25°C. compared with the very poor germination of the NPA races at the same temperature in the previous temperature must be attributed to their pre-exposure for twenty minutes to optimal germinating temperatures. The extract appeared to stimulate germination within the first twenty minutes, and once stimulated adverse temperatures did not check the process.

The type of germ tube development summarized in Table 8 gave an excellent indication of the temperature tolerance of the different accessions. Each developed typical stag-horns at 15°C., those of the race 1 accessions as a group being somewhat more pronounced than the NPA accessions. At 20°C. each race 1 accession again developed pronounced stag-horns. Stag-horn development of the NPA accessions was completely suppressed, but germ tube development was otherwise quite vigorous,

slightly branched and apparently normal. At 25°C. germ tube development of the NPA accessions was quite abnormal, very vesicular, and branching was completely inhibited. Germ-tube development of the race 1 accessions was quite vigorous and normal at this temperature, but stag-horns were suppressed.

Analysis of the Linseed Extract.

Detailed analysis of the extract was beyond the scope of the present investigations, but several experiments were carried out to determine within very broad limits the nature of the stimulating principle or principles. This involved ether extraction, charcoal adsorption and charring of the aqueous extract, and tests of the residue to determine whether the active properties of the extract had been eliminated or destroyed.

Since the residue after each treatment lacked the spore dispersing and immobilizing properties of the original extract small amounts of gelatin were added to the reconstituted solutions. A quantity slightly in excess of one-eighth per cent by weight was added to offset the slight deterioration of the spreading qualities of the gelatin induced by the salts, etc., in the solution. The ether extractions were made before gelatin had been adopted as an inert spreading agent.

Special precautions were taken to ensure that the reagents were free from toxic impurities.

Ether Extraction.

The 4% aqueous extract was shaken up with ether and the aqueous residue separated off. The process was repeated several times, fresh ether being used to ensure removal of all ether-soluble fractions from the aqueous residual solution. The residual solution was heated gently to eliminate all traces of ether, and finally made up with distilled water to the original volume. The ether-soluble fraction was added to water in an evaporating dish and the ether evaporated off slowly. This fraction was tested in its concentrated form and the excess made up with distilled water to the original concentration.

The percentage germination on the aqueous residue after extraction was lower than that obtained on distilled water. A concentrated solution of the ether-soluble fraction induced a very high percentage germination. It was most remarkable for the unusually large number of germ tubes which emerged from each spore. The tubes, however, failed to develop after emergence. At the normal concentration this fraction induced a rapid and high percentage germination, but the tubes again failed to develop. When the ether-soluble and ether-insoluble fractions were added together and made up to normal concentration the spores germinated rapidly and normally, almost as well as they did on the original extract.

It seemed obvious from this experiment that the germination stimulating properties of the linseed extract derived principally from the ether-soluble fraction. This fraction seemed to be responsible for the high percentage germination and the relatively rapid rate of germination characteristic of the linseed extract. In concentrated form it stimulated the germination process still more by inducing an unusually high number of tubes from each spore. The germ tubes failed to develop normally, however, on the ether-soluble fraction. This might have been due to impurities in the ether, though this does not seem likely in view of the precautions taken to eliminate impurities. Results tend rather to suggest that some substance or substances present in the ether-insoluble fraction were required in conjunction with the ether-soluble components to induce normal development following germination.

Charcoal Adsorption.

B.D.H. activated charcoal and charcoal obtained by sulphuric acid charring of sugar were used for this extraction. The B.D.H. charcoal had a slightly toxic effect on germination not completely eliminated by frequent leaching with boiling water. The charcoal prepared from sugar was thoroughly leached to remove all trace of acid and then heated to over 600°C. to enhance its adsorbing properties. The 4% plant extract was boiled with the charcoal for 20 minutes and the charcoal filtered off. The B.D.H. charcoal was the most efficient adsorbing agent, and yielded a clear residual

TABLE 8.

Effect of the Temperature on the Type of Germ Tube Development on 4 per cent. Linseed Extract.

Temp.	Race.	S.U. Accession Number.	Months Stored.	Type of Germ Tube Development.			
				Percentage Germination.	Activity.	Distribution.	Branching.
15° C.	1	613	9	90	Very active.	Mostly surface.	Very staggy.
	1	613	1¼	97	,, ,,	,, ,,	,, ,,
	1	507	¾	100	,, ,,	Mostly surface, slightly subsurface.	,, ,,
	1	621	½	100	,, ,,	Surface mostly, moderate amount just subsurface.	,, ,,
	1	624	1¼	98	,, ,,	Mostly surface.	,, ,,
	7	550	½	83	,, ,,	Moderate amount just subsurface.	Staggy.
	17	604	½	96	,, ,,	A lot subsurface.	Very staggy.
	17	604	11	74	,, ,,	Slight amount subsurface.	Staggy.
	13	582	1	93	,, ,,	Mostly surface.	Slightly staggy.
	18	607	½	89	,, ,,	Mostly surface.	Moderately staggy.
	15	599	½	—	——	——	——
20° C.	1	613	9	90	Very active.	Mostly surface.	Very staggy.
	1	613	1¼	97	,, ,,	,, ,,	,, ,,
	1	507	¾	100	,, ,,	,, ,,	Staggy.
	1	621	½	99	,, ,,	,, ,,	,,
	1	624	1¼	99	,, ,,	,, ,,	,,
	7	550	½	80	Normal.	A lot subsurface.	Long, slightly branched.
	17	604	½	95	,,	Mostly just subsurface.	Slightly branched.
	17	604	11	65	,,	Mostly surface.	Long, slightly branched.
	13	582	1	91	,,	A lot subsurface, quite a few long aerial hyphae.	Slightly branched.
	18	607	½	61	,,	Mostly subsurface, some aerial tubes.	Long, slightly branched.
	15	599	½	96	,,	Mostly just subsurface.	Long, slightly branched tubes.
25° C.	1	613	9	87	,,	Mostly surface.	Not staggy.
	1	613	1¼	92	,,	Plenty of surface growth.	Not staggy.
	1	507	¾	98	,,	A few long aerial hyphae, mostly surface.	Not staggy.
	1	621	½	99	,,	Quite a few long aerial hyphae, mostly surface.	Not staggy.
	1	624	1¼	96	,,	Quite a few long aerial hyphae, mostly surface.	Not staggy.
							Only slight branching.
	7	550	½	70	Abnormal inhibited.	Subsurface.	Very vesicular, no branching.
	17	604	½	87	Abnormal inhibited.	Mostly subsurface.	Very vesicular, no branching.
	17	604	11	4	Abnormal inhibited.	,, ,,	Very vesicular, no branching.
	13	582	1	93	Abnormal inhibited.	,, ,,	Very vesicular, no branching.
	18	607	½	5	Abnormal inhibited.	,, ,,	Very vesicular, no branching.
	15	599	½	97	Abnormal inhibited.	,, ,,	Very vesicular, no branching.

solution lacking the surface properties of the original solution. The residual solution after the sugar charcoal extraction was less clear and possessed some of the surface properties of the original.

The spores of the four rust accessions tested germinated much more poorly on the residual solutions from the two charcoal extractions than they did on the original extract. Addition of gelatin to the residual solutions increased the percentage

TABLE 9.
Ether Extraction of Extract.
Race 2 Accession 492.

	Germinating Medium.	Percentage Germination.	Type of Germination.
1*	Distilled water.	30	Very short germ tubes.
2†	Normal strength linseed extract (boiled 2 minutes).	95	Dense, very vigorous, staggy germ tube development.
3*	Residue of boiled extract after ether extraction.	5	Long and unbranched germ tube.
4†	Fraction extracted by ether and brought back into aqueous solution.	90	Rapid germination but germ tubes failed to elongate.
5†	3 and 4 combined.	90	Very rapid germ tube development, long, not so even as 3 or 4, and slightly branched.
6†	Concentrated solution of 4.	85	Remarkable number of germ tubes from each spore, but tubes failed to develop normally.

* Maximum estimate. Spores too mobile to count.
† Minimum estimate.

germinations, but not appreciably beyond the figures obtained on pure one-eighth per cent gelatin. The substance or substances responsible for the germination stimulating potential of the plant extract had been adsorbed by the charcoal.

Charring of the Extract.

The plant extract was evaporated to dryness in a low temperature oven and then heated to about 600°C. for an hour to eliminate all traces of organic matter. The

TABLE 10.
Charcoal Adsorption of Extract.

		Percentage Germination after 48 Hours at 15° C.				
	S.U. Accession	613	528	604	557	
	Race	1	6	17	1	
	Months stored	9	3	11	9½	
	Germinating Medium.					Average.
1	Normal linseed extract	92	75	80	52	75
2	Gelatin ⅛%	69	65	12	40	47
3*	Linseed extract residue after treatment with activated B.D.H. charcoal	30	15	2	15	16
4	3 with gelatin added..	75	51	6	35	42
5	Linseed extract residue after treatment with sugar charcoal	55	19	5	25	26
6	5 with gelatin added..	74	66	14	41	49

Germination based on count of 700 spores approximately.
* Spores too mobile on surface. Maximum estimate for 3.

residue was brought back into solution and made up to the original volume. It lacked all the surface properties of the original solution.

Eight rust accessions were used to check the germination potential of the charred extract. All of them gave very much lower germinations on the charred extract than

they did on the original. Addition of gelatin to the charred extract increased germination of six of the accessions appreciably, three of them to a level almost equal to germination on the original extract. This need not be attributed to the fact that the residue after charring together with the gelatin possessed the stimulating properties of the plant extract. It could be attributed rather to the fact that the spores had not become severely storage-hardened although they had been stored in some cases for periods of 9½ months. Particular importance attached to the results obtained with

TABLE 11.
Effect of Charring on Stimulating Properties of the Extract.

	S.U. Accession	507	557	613	496	528	550	568	604	
	Race	1	1	1	5	6	7	11	17	
	Months stored	3½	9½	9½	3½	3½	3½	3½	12	
	Germinating Medium.	Percentage Germination after 48 Hours at 15° C.								Average.
1	Normal linseed extract	95	70	84	90	84	64	69	64	78
2*	Charred linseed extract	80	10	60	50	50	4	20	2	35
3	Charred extract plus gelatin ..	91	26	79	82	63	39	55	1	55

* Maximum estimate. Other percentage germinations based on count of 700 spores approximately.

race 17. Tests of the same sample of rust from which these spores had been drawn showed consistently that they were severely storage-hardened. The charred extract with and without gelatin failed to increase germination of the race 17 spores beyond 2%, although the spores were still highly viable judging by their germination on the original extract. It could be assumed that charring of the extract had destroyed the substance or substances responsible for the germination stimulating potential of the original plant extract.

TABLE 12.
The Effect of the Germinating Medium on Type of Germ Tube Development.

Race.	S.U. Accession.	Linseed Extract.	Charred Solution.	Charred Solution and Gelatin.
1	507	Moderately staggy. Superficial growth.	Long unbranched. Aerial growth.	Long unbranched tubes. Aerial and superficial.
1	557	Very staggy. Superficial growth.	Long unbranched. Aerial growth.	Long unbranched tubes. Aerial and superficial.
1	613	Very staggy. Superficial growth.	Long unbranched. Aerial growth.	Long unbranched tubes. Aerial and superficial.
5	496	Very staggy. Superficial growth.	Long unbranched. Aerial growth.	Long unbranched tubes. Aerial and superficial.
6	528	Rather staggy. Superficial growth.	Long unbranched. Aerial growth.	Long unbranched tubes. Aerial and superficial.
7	550	Rather staggy. Moderate amount subsurface.	Long unbranched. Aerial growth.	Long unbranched tubes. Aerial and superficial.
11	568	Rather staggy. Superficial growth.	Long unbranched. Aerial growth.	Long unbranched tubes. Aerial and superficial.
17	604	Slightly staggy. Superficial growth.	Long unbranched. Aerial growth.	Long unbranched tubes. Aerial and superficial.

Preliminary Studies of Uredospore Longevity at 0°C. to 2°C.

Initial studies of uredospore longevity were based on results obtained with spores stored in a refrigerator and used for the genetical investigations of disease resistance. These results are listed in Table 13.

The results did not indicate any consistent correlation between the period of cold storage and survival of the uredospores, although there was an obvious serious falling off in viability by the end of twelve months. Some other factor or factors with time played the dominant role in determining the survival of the spore samples. Various possibilities were considered, e.g., race, spore bulk during storage, production at

different stages in the sporulation cycle, production at different times of the year, pre-storage temperatures, freezing and thawing effects in the refrigerator, etc. These possibilities are dealt with in the following section.

TABLE 13.

Date Collected.	Time Stored. (Months.)	Race, Accession and Percentage Germination.
21:11:50*	6	1. 557 60% 586 50% 2. 544 0% 548 15% 552 75%
		3. 511 0% 556 0% 4. 558 50% 578 50%
		5. 514 85% 515 70% 554 60% 7. 550 15% 8. 555 1/10%
		9. 559 80% 583 0% 10. 579 85% 11. 568 40%
		12. 580 10% 13. 582 50% 14. 584 3%
13:11:50*	6	2. 544 80% 3. 511 70% 556 80% 8. 555 70%
		9. 583 50% 14. 584 60%
23:10:51	8	2. 552 54% 560 56% 4. 518 42%
		5. 514 52% 515 53% 11. 568 57%
20:10:51	8	2. 552 59% 560 70% 548 72% 4. 518 58%
		5. 514 67% 515 60% 11. 568 75%
13:11:50	10	1. 507 50% 586 60% 2. 496 81% 544 56% 548 76% 552 88%
		4. 558 84% 578 81% 5. 515 94% 554 82% 6. 551 59%
		7. 550 74% 9. 559 1% 10. 572 83%
		11. 568 20% 12. 580 57% 13. 582 79%
24:11:50	9½	1. 586 87% 12. 580 83%
28: 7:50*	11	1. 557 30% 2. 548 ¼% 560 0% 3. 556 30%
		6. 551 50%
31: 7:50*	11	1. 557 30% 3. 556 4%
3: 8:50*	11	1. 557 50% 2. 548 25% 560 50% 3. 556 40%
		6. 551 70%
8: 8:50*	11	1. 557 30% 2. 548 30% 560 40% 3. 556 20%
		6. 551 25%
1: 5:50*	14	1. 507 15% 2. 492 30% 544 10%
		5. 496 5% 515 10%
2: 6:50*	13	1. 557 2. 552 3. 556 4. 558 5. 554
		8. 555 9. 559 All inviable.
17: 7:51	12	1. 557 34% 586 38% 2. 548 43% 552 56% 560 27%
		3. 556 12% 4. 558 44% 578 14%
		5. 514 69% 515 39% 554 18% 6. 551 ½% 9. 559 18%
		10. 579 13% 11. 568 11% 13. 582 30%
18: 8:50	14½	1. 557 0% 2. 548 1% 560 4% 3. 556 20%
15: 8:50	14½	1. 557 36% 2. 548 9% 560 ½% 3. 556 12%
11: 8:50	14½	1. 557 13% 2. 548 4% 560 1% 3. 556 8%
31: 7:50	15	1. 557 ½% 2. 548 — 560 0% 3. 556 0%
12: 4:50	14½	1. 507 1% 547 5% 2. 492 15% 548 ½% 5. 496 ½% 519 2%
		1. 507 5. 514 515 6. 528 Inviable.
26: 4:50	14	2. 512 5% 5. 496 20% 519 25% 4. 518 1%
		2. 492 6. 528 Inviable.
17:11:50	17	2. 560 12% 3. 511 1% 8. 555 18%
		1. 507 2. 548 3. 556 4. 558 578 5. 554 Inviable
		6. 551 7. 581 550 9. 559 583 10. 579 Inviable
		11. 568 12. 580 13. 582 Inviable.
13:11:50	17	1. 586 5% 2. 544 3%
		1. 507 557 2. 548 560 4. 558 5. 496 Inviable.
		6. 551 7. 550 10. 579 11. 568 Inviable.
2:11:50	17½	5. 496 Lot A 18% Lot B 16%

* Estimated percentage germination.

Race Differences.

Race 2 (accession 492) appeared to survive better than other accessions. But apart from this there were no consistent differences in the longevity of uredospores of different accessions stored in the refrigerator. Race 9 (accession 559) was one of the most viable of those tested after six months. It was one of the least viable accessions after ten months' storage. Several accessions were identified as the same race, but different accessions of the same race often differed as much in viability after prolonged storage as accessions of different races. It was obvious that a factor

or factors other than race played the dominant role in determining the viability of uredospores in cold storage.

Spore Bulk During Storage.

Small quantities of spores tended to survive better than spores stored in bulk. This is shown in results obtained with race 2 accession 492, confirmed on many occasions

TABLE 14.

Collected.	Quantity of Spores Stored.	Percentage Germination on Extract.
10: 9:51	2% of tube volume.	91
10: 9:51	20% of tube volume.	10
2:10:51	25% of tube volume.	56

Count of 500 spores approximately.

by general observation. Uredospores of accession 492 were collected on 10th September, 1951. The material was divided into two lots and stored immediately. One lot of spores occupied no more than 2% of the tube volume after corking. This lot was still 91% viable after eight months' storage. The other lot, stored in a tube of equal

TABLE 15.

Viability of Spores of Race 2 (Accession 492) Collected During a Sporulation Cycle.

Period Stored.	Date Tested.	Date of Collections of Uredospores.				
		6:10:50.	10:10:50.	13:10:50.	16:10:50.	20:10:50.
9 months ..	11: 7:51*	80%	80%	80%	80%	80%
12 months ..	17:10:51†	67%	77%	81%	83%	53%

* Minimum estimate of percentage germination.

† Count of 250 spores approximately.

size, occupied about 20% of the total tube volume. It was no more than 10% viable after the same period of storage.

But bulk storage was not always detrimental to uredospore longevity. The same accession collected three weeks later was still 56% viable after 7½ months' storage, although the spores occupied at least 25% of the total tube volume.

TABLE 16.

Viability of Spores of Race 6 (Accession 529) Collected During a Sporulation Cycle.

Period Stored.	Date Tested.	Date of Collections of Uredospores.			
		10:10:50.	13:10:50.	15:10:50.	19:10:50.
9 months ..	11: 7:51*	50%	30%	60%	70%
12 months ..	18:10:51†	25%	2%	5%	12%

* Minimum estimate of percentage germination.

† Count of 250 spores approximately.

Production at Different Stages in the Sporulation Cycle.

It was thought that the longevity of uredospores might be somewhat predetermined by the stage in the sporulation cycle during which they were produced, but this was not supported by results obtained with races 2, 6 and 7. Uredospores of these races had been collected at two- to four-day intervals during a single vigorous sporulation cycle, and were tested for viability after nine and twelve months' cold storage. There was no consistent correlation between the time in the sporulation cycle when the spores were produced, and their viability after nine and twelve months' cold storage. Sometimes spores produced early survived better than spores produced later. Sometimes

the reverse was found. It could only be concluded that spores could remain viable for considerable periods whether produced early, late or part way through the sporulation cycle.

Production at Different Seasons.

Uredospores collected during winter often became inviable relatively quickly. It seemed possible that spores produced during winter lacked the capacity for survival of spores produced in spring and summer. But examination of results collected over several years showed that spores produced during winter could remain viable as long

TABLE 17.
Viability of Spores of Race 7 (Accession 550) Collected During a Sporulation Cycle.

Period Stored.	Date Tested.	Date of Collection of Uredospores.				
		28:8:50.	30:8:50.	1:9:50.	4:9:50.	8:9:50.
9 months ..	11: 7:51*	—	60%	20%	40%	30%
12 months ..	18:10:51†	18%	47%	4%	22%	21%

* Minimum estimate percentage germination.
† Count of 250 spores approximately.

as spores produced at other times of the year. Nine of the ten races collected in mid-July were still moderately to highly viable after twelve months (Table 18A). Spores of race 2 (accession 492) collected in early July were as viable after 13½ months' storage as spores collected in warmer months and stored for shorter periods (Table 18B).

Prestorage for Several Days at Temperatures up to 20°C.

Uredospores of the different accessions were collected in general at two- to four-day intervals. Temperatures in the glasshouse varied considerably from day to day, and during each day. It seemed possible that these random variations in temperature might affect the longevity potential of spores collected on different days, depending on the period the spores were allowed to remain *in situ* prior to collection. The possibility

TABLE 18A.
Percentage Survival of 10 Races after 12 Months' Cold Storage.

Collected.	Percentage Germination of Accessions on 4% Extract after 48 Hours. (Count of 400 spores approximately.)						Period Stored.
17:7:51	Race 1	Ac557 34%	Ac586 38%				12 months.
	Race 2	Ac548 43%	Ac552 56%	Ac560 27%			
	Race 3	Ac556 12%					
	Race 4	Ac558 44%	Ac578 14%				
	Race 5	Ac514 69%	Ac515 39%	Ac554 18%			
	Race 6	Ac551 ½%					
	Race 9	Ac559 18%					
	Race 10	Ac579 13%					
	Race 11	Ac568 11%					
	Race 13	Ac582 30%					

was checked by exposing freshly collected spores of race 2 (accession 492) to temperatures of 8°C. to 20°C. for varying periods before transferring them to the refrigerator. Check samples were placed in the refrigerator without prior treatment.

It appeared from the results that exposure of spores to temperatures up to about 20°C. for periods of about ten days did not seriously affect the capacity of spores to retain their viability in cold storage. Since the mean daily temperature rarely exceeded 20°C. during those months when rusts were cultured in the glasshouse, and since spores were seldom collected at greater than four-day intervals, it seemd highly improbable that temperature conditions prevalent during the period of spore formation in any way affected the potential longevity of spores in cold storage.

Alternate Freezing and Thawing.

Freshly collected uredospores, and uredospores kept in cold storage for three months were subjected to six cycles of freezing and thawing to determine the immediate effect, if any, on viability. A parallel series of tests was carried out for each of three races, races 1, 2 and 6. The spores were chilled in the tray beneath the cold box

TABLE 18B.

Viability of Spores of Race 2 (Accession 492) Collected at Different Times of the Year 1950.

Date Collected.	6:12.	4:10.	23:9:1950.						25:8.	30:8.	29:8.	15:8.	8:7.
Months stored ..	1½	3½	4½						5	5	5	5½	6½
Percentage germination* ..	90	95	90	90	90	90	90	90	95	95	95	85	85
Months stored ..	8½	10½	11	11	11	11	11	11	12	12	12	12½	13½
Percentage germination† ..	77	85	85	68	87	90	63	—	81	85	79	51	79

* Minimum estimate of percentage germination.

† Count of 300 spores approximately.

of the refrigerator at a temperature of 0°C. to 2°C. for half an hour. They were then removed and left at room temperature (approximately 15°C.) for another half-hour. A sample was taken off for determination of percentage germination on extract before returning the material for a further cycle. After six such cycles none of the races freshly collected or stored for three months showed any detectable reduction of viability when 600 spores were counted.

The process was continued for another six cycles, during which the half-hour period was lengthened to more than an hour. At the conclusion of this series there was still no detectable reduction of viability.

TABLE 19.

Effect of Pre-Storage at Higher Temperatures, Race 2.

Temperature.	Check Sample.	Days Stored at the Temperatures Listed.				
		4	9	14	18	21
13°–15° C. ..	90%	—	80%	80%	70%	60%
18°–20° C. ..	95%	85%	75%	35%	—	1%

Estimated percentage germination after four months' cold storage.

It was concluded that minor fluctuations of temperature in the refrigerator caused by daily opening of the refrigerator door and major fluctuations which occurred three to four times each year during defrosting would probably not have any serious effect on the viability of the stored spores.

A Note on Uredospores Produced in the Fluorescent Light Room.

Uredospores collected during November, 1950, were produced by plants growing indoors under fluorescent lighting. The excellent viability of these spores after 9½ to 10 months' storage showed that spores produced under artificial light had much the same potential longevity as spores produced under natural light.

Summary.

The preliminary studies showed that most if not every race could remain viable for at least a year, although unknown factors often caused them to become inviable earlier. The percentage survival after a year ranged from 0% to almost 90%, but could not be correlated with race differences, the time of the year, or stage in the sporulation cycle when the spores were produced. There was no evidence to suggest

that periodic fluctuations in the refrigerator temperature or exposure of spores to temperatures up to 18°C. for several days in any way significantly affected the capacity of spores to remain viable in cold storage. Spores stored in bulk often became inviable more quickly than spores stored in small amounts, but this was due to some other factor than mere spore bulk. Some other major factor or factors were responsible for the rather unpredictable survival of the spores in the refrigerator.

Spores stored in the refrigerator during the early stages of these investigations probably varied considerably in their moisture content (at least for the first few weeks) according to the humidity prevalent during their production in the glasshouse. Spores collected during rainy weather were noticeably moister than spores collected in fine weather. Since they were transferred into tubes and corked and stored almost immediately after collection they had little opportunity to lose any excess moisture.

TABLE 20.

Effect of Pre-Storage at Higher Temperatures, Race 2.

Date Collected.	Period of Cold Storage.	Check Sample.	Days Stored at 18° C.			Days Stored at 8° C.	
			3	7	11	7	11
4:12:50	2 months*	90%	90%	90%	85%	90%	85%
	9 months†	84%	85%	89%	81%	85%	80%

Date Collected.	Period of Cold Storage.	Check Sample.	Days Stored at 18° C.		
			3	6	10
1:12:50	2 months*	85%	85%	80%	70%
	9 months†	78%	74%	68%	68%

* Minimum estimate.

† Count of 250 spores approximately.

Once this risk had been realized, spores stored in any bulk were allowed to dry out in the tubes before corking or stoppered with cotton wool plugs. Careful observations were subsequently made of spores collected in wet weather and stored immediately. Drops of moisture frequently appeared inside the tube just below the cork. The moisture usually disappeared after a couple of weeks, but it is certain from the results obtained later at 75% relative humidity in a carefully controlled experiment that even at the low temperatures prevailing in the refrigerator there could have been a serious loss of viability before the excess moisture had been transpired off by the spores and escaped from the tubes. The volume of the tube and tightness of fit of the cork would play an important role in determining the period during which the humidity remained at a critically high level. These findings explain the common observations that spores collected in winter (the rainy season in Sydney) often, but not always, lost their viability sooner than spores collected in summer, and that spores stored in bulk often became inviable sooner than spores stored in small quantities.

Unless precautions are taken to ensure that the spores are reduced to the same approximate moisture content at the start of longevity studies and stored under conditions preventing local build-up of humidity around the spores in the storage tubes, undetected variations in humidity are likely to obscure the effects of controlled factors such as race and temperature. This was made very clear by the highly capricious percentage survival of the races studied during the early investigations of uredospore longevity at Sydney.

It is fairly certain that precautions taken during the final studies of longevity detailed in the next subsection eliminated this risk. The use of linseed extract should also have eliminated the equally grave risk arising from the capricious and submaximum germination of *M. lini* uredospores on the common germinating media.

Uredospore Longevity of Three Races at Carefully Controlled Temperatures and Humidities.

Experimental Methods and Materials.

The project was commenced in May, 1952, when it was decided to determine the storage capacity of three races, 1, 2 and 6, at three humidities, 25%, 50% and 75% relative humidity, and three temperatures, 0°C., 3·5°C. and 10°C. in all combinations.

The humidity was regulated in medium-sized desiccators with sulphuric acid of known specific gravity (Maclean and Cook). The acid was made up to the requisite specific gravity with distilled water by weight and checked with a hydrometer. Temperature control facilities were very kindly made available by the C.S.I.R.O. Division of Food Preservation and Canning, Homebush. Under these conditions temperatures did not fluctuate by more than ±0·2°C. during the course of the experiment.

The uredospores of the three races were built up under identical conditions on fully susceptible varieties in the glasshouse during mid-winter. Mild sunny weather prevailed from the date of infection to completion of sporulation. The temperature recorded on a thermograph showed a diurnal range, fluctuating from a maximum of 68°F. to 82°F. at 2 p.m. to a minimum of 46°F. to 56°F. about 8 a.m. Except for a few brief cloudy periods the infected plants were exposed to uninterrupted direct sunlight in the glasshouse from sunrise to sunset. There was no artificial lighting, but temperatures were kept slightly above outdoor temperatures at night to ensure vigorous sporulation.

The spores were collected at three-day intervals between 5.30 p.m. and 6 p.m. The first collection was not retained for the studies, but the next four collections were kept separately from the time of collection in small glass phials, stoppered lightly with cotton wool, and placed in a desiccator at 50% relative humidity at 3°C. Spores were collected only during the period of vigorous sporulation. After the last collection had been stored at 50% RH for 24 hours the spores were prepared for storage under the conditions mentioned above. A bulk sample of each race was prepared by mixing together equal quantities of material from the four collections. Thorough mixing was achieved when the bulk sample was sieved through a very fine mesh wire screen to remove all traces of organic matter which might accidentally have been collected with the rust.

The spores were set up in small glass tubes specially hand-made from narrow glass tubing about one-twelfth inch internal diameter. Each tube, about one inch in length, was identified for race, temperature and humidity with small colour patches painted in a vertical series of three spots. They were left in a well-aired position for several weeks after painting to eliminate the risk of fumes affecting viability during storage.

Equal amounts of rust were added to each tube to a depth of about one-tenth inch. The tubes were then mounted unstoppered in wooden blocks bored with the requisite number of holes to a depth of about half an inch. These blocks had been soaked in liquid paraffin some time previously to eliminate risk of swelling or mildew development at the higher humidity. Eighteen tubes of each race were mounted in each of nine such blocks, one block for each combination of temperature and humidity.

The blocks were mounted on glass trays inside the desiccators to avoid any risk of sulphuric acid splashing onto the spores or onto the wooden blocks.

The desiccators were set up at the given temperatures at Homebush, and arrangements were made for samples to be sent in for germination tests at Sydney University at monthly intervals.

These samples were set up immediately on receipt on 4% host extract and kept in the dark at 15°C. in closed small petri dishes for at least 40 hours before germination counts were made. The spores were examined under the high power of the microscope undisturbed on the extract. A minimum of 500 spores was counted for each sample. In general about 600 spores were counted. The studies were continued for 13 months.

Experimental Results.

The results have been graphed and summarized in three sections.

Graphs 7 to 15 give the viability curves of each race under the nine different storage conditions, to highlight race differences. Graphs 1 to 6 were plotted from the averaged percentage germination of the three races at each storage condition.

TABLE 21.

Percentage Survival of Uredospores of Races 1, 2 and 6 Stored at 0° C., 3·5° C. and 10° C. and at 25%, 50% and 75% RH (in all Combinations) after Storage for One to 13 Months.

Relative Humidity.	Months Stored.	0° C.			3·5° C.			10° C.		
		Race 1	Race 6	Race 2	Race 1	Race 6	Race 2	Race 1	Race 6	Race 2
25%	1	95	97	97	95	99	98	91	99	99
	2	94	96	97	96	97	95	92	96	96
	3	84	92	90	82	94	90	80	92	87
	4	71	83	74	58	84	69	35	41	23
	5	47	73	45	44	60	37	16	29	1·6
	6	28	55*	26	5	34	12	0·8	0·9	0·1
	7	23	49	18	7	27	6	0	0	0
	8	8	27	8	2·1	6	1·2	0	0	0
	10	4	12	0·8	0·1	0·3	0·1			
	11	1·3	3·4	1·5	0	0	0			
	12	1·4	2·9	0·9	0	0	0			
	13	0	0·1	0·0						
50%	1	97	98	97	92	97	97	87	98	99
	2	97	97	97	95	99	98	90	96	99
	3	94	98	95	72	96	97	62	83	95
	4	82	95	93	69	94	93	38	29	50
	4½	—	—	—	—	—	—	22	5	34
	5	86	90	91	57	87	94	6	2·5	21
	6	85	90	86	54	72	85	1·8	0·2	0·8
	7	81	88	87	54	66	84	0	T.	T.
	8	55	79	80	13	17	42	0	0	0
	10	50	61	76	4·4	2·4	12	0	0	0
	11	72	72	76	5	1·3	6			
	12	58	80	80	2·7	0·3	3·2			
	13	44	42	40	1	T.	T.			
75%	1	90	93	91	52	64	65	1·4	15	20
	2	58	65	65	1·5	24	28	0	0	0·1
	3	19	26	26	T.	3·6	13	0	0	0
	4	3·7	13	11	0	0·1	0·3	0	0	0
	4½	4·7	11	17						
	5	1·5	10	10	0	0	0			
	6	0	7	11	0	0	0			
	7	T.	6	10						
	8		Lost							
	10	0	0	0						

* Difficult to assess percentage germination of this lot owing to excessive clumping together of the spores.

Germination above 5% given to nearest 1%. Germination between 1% and 5% given to nearest 0·1%. Germination below 1% given to nearest 0·1%.

"T."=Trace germination less than 0·1%.

Graphs 1 to 3 highlight the humidity effect on viability at each temperature. Graphs 4 to 6 were prepared to highlight the temperature effect on viability at each humidity.

Effect of Humidity on the Longevity of Uredospores.

0°C.

The effect of humidity on uredospore longevity was particularly marked at this temperature. The spores retained a high level of viability for the first month at 75% RH, but deteriorated very rapidly at an almost linear rate during the next two months to an average of 24% viability. By the end of the fourth, viability had entered the final lag phase terminating between the seventh and eighth months of storage.

The spores remained highly viable for the first three months at the lower humidities, although survival was slightly better at 50% RH. After this time spores held at 25% RH began to deteriorate at an almost linear rate to 14% viability at the end of eight months. There was a prolonged lag phase terminating after about thirteen months of storage.

A high level of viability was maintained at 50% RH for the first twelve months. Results at the end of thirteen months suggested that the spores had entered the intermediate phase of rapid decline. But in view of the rather capricious germination obtained earlier at eight and ten months' storage it is possible that the spores were more viable at the end of thirteen months than the germination figures indicated.

3·5°C.

There was marked deterioration within the first month at 75% RH. This deterioration appeared to proceed at a linear rate from the commencement of storage until at least the end of the second month of storage, when viability had fallen to 18%. Viability entered a brief lag phase terminating before the end of the fifth month of storage.

Spores retained their viability equally well at 25% RH and 50% RH for the first three months. After this period spores held at 25% RH began to lose viability at an almost linear rate until the end of the sixth month, when the three races averaged 17% viability. The curve then passed into a slight lag phase terminating before the end of eleven months. Spores kept at 50% RH did not appear to enter the intermediate phase until the end of the eighth month of storage and fell from 68% viability to 24% viability over the next month. There was a moderately long final lag phase and the spores were virtually inviable after thirteen months' storage.

10°C.

Spores held at 75% RH were only 12% viable at the end of the first month and inviable by the end of two months. They retained a high level of viability for the first three months at the lower humidities, but entered the intermediate phase of very rapid decline shortly after this. They were inviable at both humidities by the end of seven months. Although there appeared to be only a slight difference between the viability curves at 50% and 25% RH results from the individual races indicated interesting differences.

Effect of Temperature on the Longevity of Uredospores.

25% RH.

Spores retained much the same level of viability at each temperature for the first three months, then entered the intermediate phase of viabiltiy and deteriorated rapidly. The rate of deterioration increased with increasing temperature. Spores held at 10°C. were virtually inviable by the end of six months. There was no obvious final lag phase. The spores were virtually inviable after ten months at 3·5°C. and after twelve months at 0°C.

50% RH.

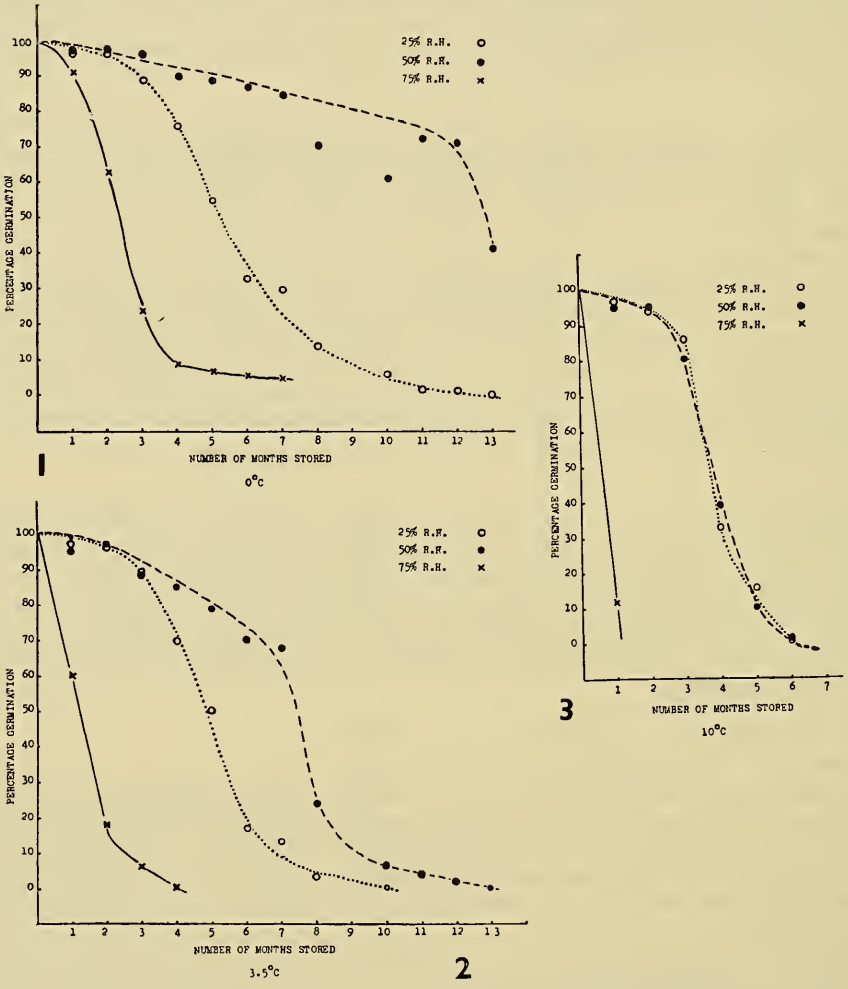
The spores survived equally well at each temperature for the first two months. From the end of the third month differences between the viability curves at each temperature became increasingly pronounced. The spores were almost inviable at the end of six months at 10°C. and after thirteen months at 3·5°C. They were still approximately 40% viable after thirteen months at 0°C. As at 25% RH spores held at 10°C. showed no obvious final lag phase, while spores held at 3·5°C. entered a final lag phase of several months' duration.

75% RH.

Spores held at 10°C. and 3·5°C. entered the intermediate phase within the first month. By the end of the first month spores held at 10°C. had virtually entered the final lag phase, but spores held at 3·5°C. were less than half-way through the intermediate phase.

By the end of two months spores held at 10°C. were virtually inviable and spores held at 3·5°C. had virtually entered the final lag phase extending until shortly after the end of the fourth month.

Spores held at 0°C. retained a high level of viability for the first month, but soon entered the intermediate phase which gave way to the final lag phase between the third and fourth months. The final lag phase was considerably extended at this temperature, terminating some time after the end of the seventh month.



Race Differences.

Since the relative survival of the three races was remarkably constant at each of the humidities irrespective of the temperature, the humidity-race interaction appeared to be rather significant. Race differences have therefore been summarized according to the humidity of the different storage conditions.

25% RH.

The relative survival of the three races remained remarkably constant at each temperature at each stage of the viability curve. During the initial lag phase race 1 deteriorated faster than races 2 and 6. The last two races maintained much the same level of viability for the first two months. By the end of the third month each race was on the verge of the intermediate phase. During the following intermediate phase

race 6 survived much better than the other two races. This was indicated not only by its greater viability at each test, but also by its more normal spore colour. The averaged viability of races 1 and 2 at the lower temperatures was for four months 20% lower than the viability of race 6, reaching a maximum difference of 29% after four months and six months at 3·5°C. and 0°C. respectively. The superior survival was maintained until the spores of each race had already entered the final lag phase. Each race, however, became inviable at much the same time.

Deterioration of spore colour was characteristic of these storage conditions. Spore colour did not deteriorate under the other humidities or under any other set of storage conditions during the current studies of longevity, etc. This deterioration was obvious from the third month, and became progressively more pronounced until the spores had become inviable and colourless. The spores were studied under the

TABLE 22.
Uredospore Colour after Storage at 25% RH at Three Temperatures.

Temperature.	Race.	Period Stored.				
		6 Months.	8 Months.	10 Months.	11 Months.	13 Months.
0° C.	1	Capucine orange.	Capucine orange to capucine buff.	Capucine buff.	Pale yellow orange.	Very pale yellow orange.
	6	Orange.	Capucine yellow.	Capucine orange.	Capucine orange to orange buff.	Capucine buff.
	2	Capucine orange.	Capucine buff.	Capucine buff to pale yellow orange.	Pale yellow orange.	Pale orange yellow.
3·5° C.	1	Capucine buff, capucine orange.	Capucine buff.	Pale yellow orange.	Pale yellow orange.	Very pale yellow orange.
	6	Mikado orange.	Capucine yellow.	Orange buff.	Capucine buff.	Capucine buff to pale yellow orange.
	2	Capucine orange.	Capucine buff.	Pale yellow orange.	Pale yellow orange.	Pale orange yellow.
10° C.	1	Capucine orange to capucine buff.	Pale orange yellow.	Pale orange yellow.	Pale orange yellow.	Very pale orange yellow.
	6	——	Orange buff.	Orange buff to light orange yellow.	Capucine buff.	Pale orange yellow.
	2	——	Pale orange yellow.	Pale orange yellow.	Pale orange yellow.	Very pale orange yellow.

Uredospores stored at 50% and 75% relative humidities at all three temperatures retained their original colour for at least 10 months. Spores kept at 75% RH then showed slight changes. Spores at 50% RH were little affected. Original colour: cadmium orange. (Colour description based on Ridgway's "Color Standards and Color Nomenclature", Plate III.)

microscope. Owing to the gradual gradation of colour between individual spores it was impossible to determine accurately the number of colourless spores. But the figures obtained showed that race 6 had a much lower percentage of colourless spores than the other races. Careful checks under the microscope during the early stages of germination failed to discover any colourless spores which had germinated.

50% RH.

Comparison of the relative survival of the three races at the different temperatures at 50% RH was complicated by the fact that spores stored at 0°C. had only just entered the intermediate phase when the studies were concluded. Germination particularly of race 1 was often highly capricious.

Race 1 appeared to deteriorate faster than the other races during the initial lag phase and the early part of the intermediate phase. Race 6, which maintained much the same level of viability as race 2 during the initial lag phase, was appreciably less viable than race 2 during the intermediate phase, and slightly less viable than race 1 during the final lag phase. Race 2 was obviously better adapted than the other races

for survival at this humidity and was considerably more viable than the other races in the final intermediate phase from the end of the fourth month at 10°C. and from the end of the fifth month at 3·5°C.

75% RH.

The rapid deterioration of all races at this humidity obscured race differences. But the relative survival of the three races paralleled the results at 50% RH. Race 1 deteriorated most rapidly, race 2 the least rapidly of the three races. Races 2 and 6 on the whole behaved remarkably alike at each temperature, and were appreciably more

TABLE 23.

Percentage of Colourless Spores after Storage under Conditions Listed for Varying Periods.

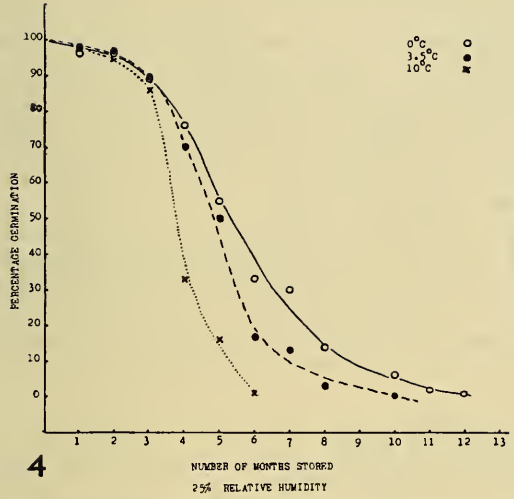
Temperature.	Humidity.	Race.	Months Stored.		
			4*	5	6
0° C.	25%	1	25	31	45
		6	10	12	22
		2	15	28	51
	50%	1	0	0	0
		6	0	0	0
		2	0	0	0
	75%	1	0	0	0
		6	0	0	0
		2	0	0	0
3·5° C.	25%	1	25	32	61
		6	10	15	23
		2	20	30	50
	50%	1	0	0	0
		6	0	0	0
		2	0	0	0
	75%	1	0	0	0
		6	0	0	0
		2	0	0	0
10° C.	25%	1	30	42	65
		6	10	17	25
		2	20	30	68
	50%	1	0	0	0
		6	0	0	0
		2	0	0	0
	75%	1	0	0	0
		6	0	0	0
		2	0	0	0

* Estimate. The rest obtained from count of 400 spores approximately.

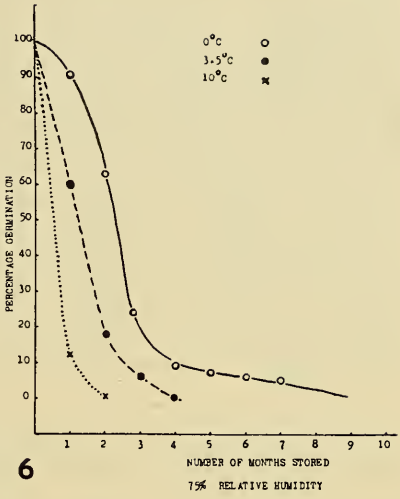
viable than race 1 at each test. The differences in percentage survival at any time of race 1 on the one hand and races 2 and 6 on the other were greatest at the two higher temperatures, but the difference in longevity of the two groups was most pronounced at 0°C., the spores of race 1 being virtually inviable after five months and the spores of the other two races still appreciably viable after seven months at 0°C.

Notes on Investigations of Uredospore Respiration.

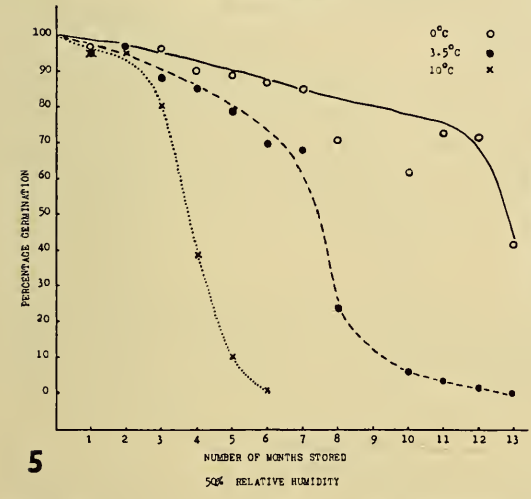
A series of experiments was carried out using the Warburg apparatus to determine the nature of the respiration curves associated with different phases of uredospore germination. It was anticipated that these studies would show characteristic differences between the respiration curves of spores sown on water, gelatin and extract and


0°c
3.5°c
10°c
PERCENTAGE GERMINATION
NUMBER OF MONTHS STORED
25% RELATIVE HUMIDITY

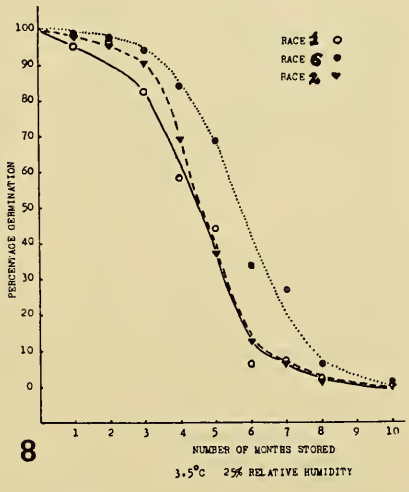
4


0°c
3.5°c
10°c
PERCENTAGE GERMINATION
NUMBER OF MONTHS STORED
75% RELATIVE HUMIDITY

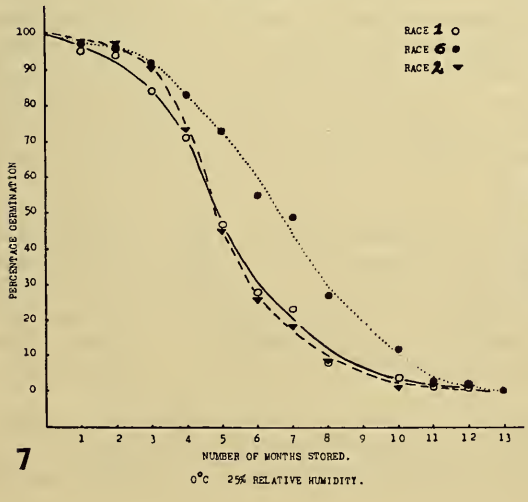
6


0°c
3.5°c
10°c
PERCENTAGE GERMINATION
NUMBER OF MONTHS STORED
50% RELATIVE HUMIDITY

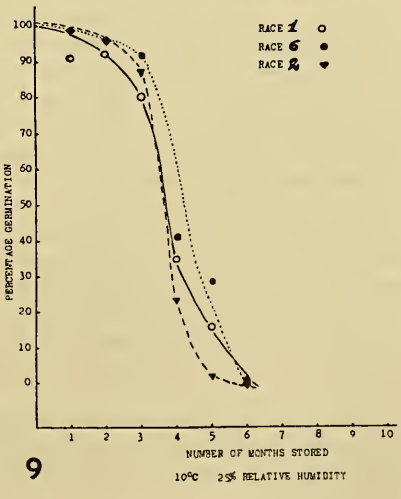
5


RACE 1
RACE 6
RACE 2
PERCENTAGE GERMINATION
NUMBER OF MONTHS STORED
3.5°c 25% RELATIVE HUMIDITY

8


RACE 1
RACE 6
RACE 2
PERCENTAGE GERMINATION
NUMBER OF MONTHS STORED.
0°c 25% RELATIVE HUMIDITY.

7


RACE 1
RACE 6
RACE 2
PERCENTAGE GERMINATION
NUMBER OF MONTHS STORED
10°c 25% RELATIVE HUMIDITY

9

possibly between Punjab-attacking and non-Punjab-attacking races. Despite many modifications of technique, spores set up under otherwise optimal conditions failed to germinate in the Warburg flasks. Spores gave a slight percentage germination when sown on solution in very small quantities, but such quantities were inadequate to give an appreciable respiration reading. Spores sown in bulk in a confined space may produce an excessive amount of germination inhibiting substances or induce local anaerobic conditions. Under normal conditions spores exposed to the air obtain an ample oxygen supply and any deleterious substances are probably destroyed by oxidation or rapidly diluted in the larger volume of solution.

Experiments were then carried out to determine whether dry and moistened spores of Punjab-attacking race 1 accessions differed in their non-germination respiration curves from non-Punjab-attacking races. It seemed probable that the difference in temperature tolerance of Punjab and non-Punjab-attacking races would be reflected in different curves in the temperature range 15°C. to 35°C., but repeated comparative studies failed to determine any difference between the two race groups.

TABLE 24.

Uredospore Respiration Studies.

Showing the Rate of Uptake of Oxygen by 0·7 Gramme of Race 1 Uredospores in a Saturated Atmosphere and in Aqueous Linseed Extract.

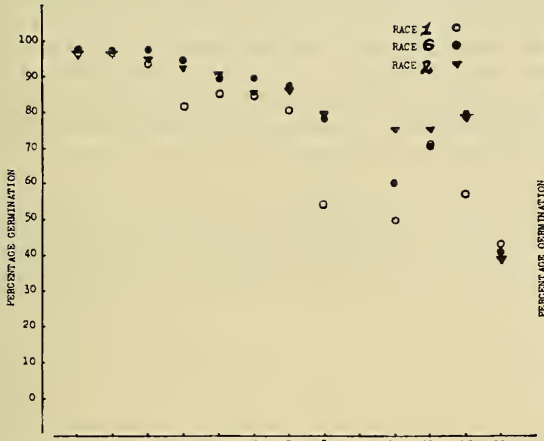
Period in minutes since commencement of experiment	0	20	40	60	70	80*	90	97·5	105	110	115	118
µl. of oxygen absorbed by uredospores since 0 time	Set at 0	47	97·4	149·7	174	205	236	456	724	1195	1386	1657
Check extract		4·7	6·3	9·4	6·3	10·9	12·5					
Period in minutes since commencement of experiment	155	160	165	170	175	255	260	270	280	290	300	310
µl. of oxygen absorbed..	Reset at 0	172	355	485	765	Reset at 0	33	52·3	64·5	66	71·5	76·5

* Extract tipped at 80 minutes.

Attempts were then made to study the respiration of the germ tubes of the two race groups. Spores were germinated in petri dishes on extract. The weft of tubes formed after 24 hours was floated off into a large container of water, washed gently and transferred without distortion onto 7 cm. filter paper. The paper was lightly rolled into a scroll. Six such papers were inserted into a Warburg flask to obtain a measurable respiration. Respiration was studied over the range 25°C. to 35°C. The results failed to reveal any significant difference between the Punjab-attacking and non-Punjab-attacking races.

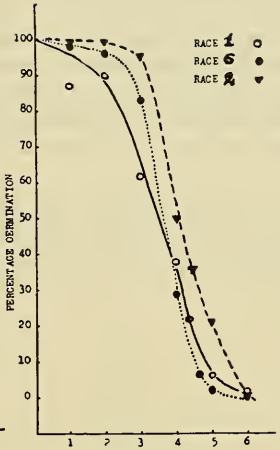
Results from one of the early experiments are illustrated in Graph 16. 0·70 gramme of uredospores of race 1 were set up in a Warburg flask to which KOH had been added in the central cup and 1 ml. of linseed extract in the side arm. The spores had been stored for a month and were 80% to 90% viable. A check flask was set up without spores to determine possible respiration of bacteria in the extract, and two control flasks were also prepared to determine change in atmospheric pressure. The temperature of the waterbath was maintained at 27°C. The usual procedures were followed.

The respiration rate of the dry spores in a saturated atmosphere was measured for 80 minutes. The extract was then tipped onto the spores and the rotating arm set in motion to disperse the spores with the solution. The results are given in Table 24 and Graph 16.



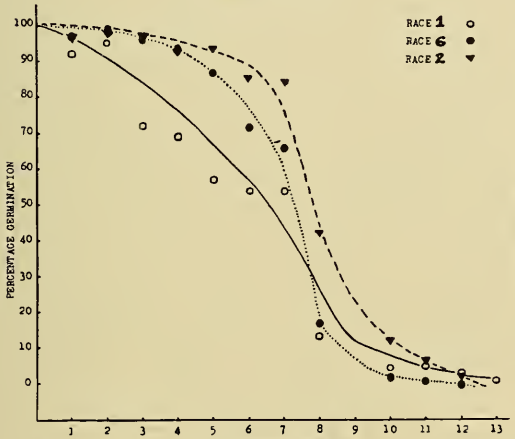
10

0°C 50% RELATIVE HUMIDITY



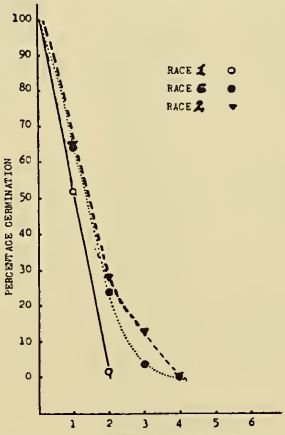
12

10°C 50% RELATIVE HUMIDITY



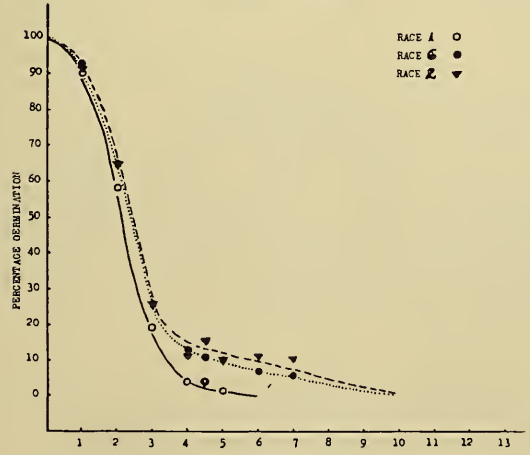
11

3.5°C 50% RELATIVE HUMIDITY



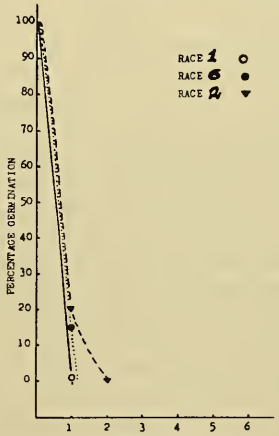
14

3.5°C 75% RELATIVE HUMIDITY



13

0°C 75% RELATIVE HUMIDITY



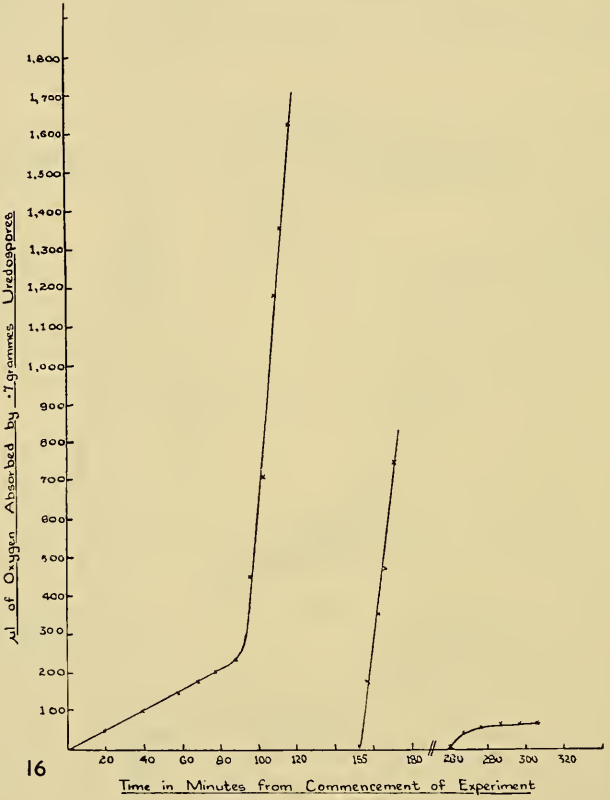
15

10°C 75% RELATIVE HUMIDITY

Respiration was extremely constant in a saturated atmosphere and the spores were absorbing oxygen at the rate of approximately 2·5 μl. per minute. Within 20 minutes of tipping the extract the rate had increased to an average uptake of 66·2 μl. of oxygen per minute. Measurements were interrupted after 118 minutes and resumed at 155 minutes. Further readings taken until 175 minutes showed that the spores were still respiring rapidly at a slightly reduced rate. Readings were again interrupted. By the time they were resumed at 260 minutes respiration had almost ceased.

Summary and Discussion.

Uredospores survived longer at 0°C. than they did at higher temperatures in humidities of 25% RH, 50% RH and 75% RH. They survived longer at 50% RH at temperatures of 0°C. to 10°C. than they did at the lower or higher humidities.



16

Uredospores stored at 0°C. and 50% RH remained highly viable for 12 months and were still very viable after 13 months, though by this stage they appeared to have entered the phase of rapid deterioration of viability. In view of the marked humidity effects at 0°C. and the marked effect of temperature at 50% RH it seems highly probable that still more favourable storage conditions could be determined by experimenting with different combinations of temperature and humidity about a mean of 0°C. and 50% RH respectively.

The very adverse effect of 75% RH on spore longevity stresses the need for keeping humidity low, especially at higher temperatures. The very capricious survival of the spore samples tested in the earlier experiments can almost certainly be attributed to the effects of high humidity inside the spore-storage phials. Spores collected during dull rainy weather, most prevalent in winter, have a higher than usual moisture content. It was discovered during the course of the final tests that spore samples collected under conditions of high humidity and stored in bulk in

cork-stoppered phials often condensed small droplets of water inside the container. This moisture disappeared within or shortly after a week. Serious deterioration would undoubtedly occur under these conditions, the degree of deterioration probably being directly proportional to the time of exposure to such humidities. Since this would be determined by such highly variable factors as the original moisture content of the spores, the spore volume and tube volume, and tightness of fit of the stopper variation between samples would be considerable. This would certainly explain why spores collected in winter and spores stored in considerable bulk usually deteriorated faster than spores collected in spring or summer and stored in small quantities.

Low relative humidity had a decidedly deleterious effect on uredospore longevity at 0°C. and 3·5°C. This effect, judging by the degree of deterioration of spore colour, was just as pronounced at 10°C. But at this temperature spores of race 6 and to a lesser degree spores of race 1 survived better at 25% RH than at 50% RH. Low humidity caused a deterioration of spore colour, destroying during the process some substance or substances responsible for normal germination. These substances may have been part of the colour complex or may have been destroyed simultaneously with these substances. Normal germination was accompanied by a gradual disappearance of spore colour. Inviable spores retained their colour for long periods after viable spores of the same sample germinated and became colourless. Abnormally germinating spores commonly extruded the orange pigment into the germ tubes, but no trace of orange pigment was ever found in the germ tubes of normally germinating spores. Spores which had become colourless during storage at 25% RH appeared to be totally inviable. The colour substance or substances tolerated a wide range of conditions and were normally not destroyed during storage. They were therefore rarely a limiting factor in the process of germination.

The rate of deterioration of the substances did not seem to be much affected by temperature. At none of the temperatures was deterioration of colour or viability marked prior to the end of the third month. But after this period there was rapid deterioration, the rate increasing slightly with increasing temperatures.

The better survival of spores of races 1 and 6 at 25% RH as against 50% RH at 10°C. was particularly interesting. It hardly seemed likely in view of the pronounced colour deterioration at 10°C. that this temperature checked the adverse effects of the lowest humidity. It seemed rather that the lowest humidity countered at another level the adverse effects of the highest temperature.

Under most storage conditions loss of viability probably resulted from a fall-off in the energy-rich reserves following normal resting respiration. The rate of deterioration would certainly have increased with rising temperature and rising humidity. Low humidity probably countered this deterioration, permitting the spores to maintain a higher level of energy-rich substances for a longer period than was possible at 50% RH. The existence of this reserve, however, became irrelevant for the maintenance of viability once the viability-determining colour complex had dropped below the critical level for maintenance of normal germination. In the case of race 1, and more especially race 6, deterioration of the colour substance was probably sufficiently delayed for spores held at 25% RH to gain a slight survival advantage over spores stored at 50% RH. But this advantage was soon cancelled out and spores of both races became inviable at much the same time at both humidities. Race 2 reacted differently. At all stages of the intermediate phase of viability its spores were appreciably more viable at 50% RH than at 25% RH. Since it is most unlikely that different processes of deterioration occurred with the different races, the following seems to be the most logical explanation. Race 2 possessed a higher level of respirable reserves than races 1 and 6. This seems to be clearly indicated by a comparison of the viability curves of this race and the other two races at the higher humidities. On the other hand, race 2 deteriorated much more rapidly than race 6 and slightly more rapidly than race 1 at 25% RH. These two factors would militate against 25% RH exerting a positive influence on the maintenance of respirable reserves before deterioration of the colour substance began to undermine viability of race 2 spores.

It is consistent with the data to assume that two different processes of deterioration operated at 25% RH. The one process was conditioned by the low humidity, and during the intermediate and final lag phase, but not the initial lag phase, was slightly accentuated by rising temperatures. It affected a substance or substances which comprised or were part of the complex responsible for spore colour. The other process of deterioration was probably directly correlated with spore respiration and was stimulated by rising temperature and humidity. At 50% RH and 75% RH loss of viability was probably due to the latter process only.

It would be interesting in future experiments to determine the effect of humidities slightly below and slightly above 25% RH on the longevity of spores stored at 10°C. It is probable that lower humidity would increase the rate of deterioration of the colour viability substance and cancel out the beneficial effects on spore respirable reserves before any benefit to viability might accrue to the spores. On the other hand, a slight rise in humidity above 25% RH might swing the balance in favour of longer survival of all races at 10°C. at such a humidity as against a humidity of 50% RH. Increase in temperature above 10°C. should favour greater longevity at 25% RH than 50% RH, since this should cause deterioration of the respirable reserves to become a limiting factor in germination before the colour complex had deteriorated below its critical level for maintenance of normal germination, assuming that a rise in temperature would have a more pronounced effect on spore respiration than on deterioration of the colour complex, since deterioration of spore colour is determined by humidity rather than temperature.

Although races did not differ greatly in their longevity under a wide range of storage conditions they did not behave identically. Race differences seemed to be correlated with humidity rather than temperature effects. The most significant differences were observed at 25% RH. Race 6 exhibited a remarkable tolerance to the adverse effects of this humidity on spore colour and viability. Race 1 deteriorated faster than the other two races under most conditions of storage. It may have had a lower respirable reserve than the other races. Race 2 deteriorated less rapidly and remained viable slightly longer than the other races at the higher humidities. It may have carried a greater reserve of respirable products. On the whole the races differed only slightly in the over-all nature of their viability curves. They generally entered the intermediate phase of rapid decline as a group and became inviable at much the same time. There is certainly a need to use pure races in determining the effect of different storage conditions on uredospore viability, especially during the intermediate phase of viability. But the results given by any one race should be fairly typical of the species as a whole.

The lower the temperature in the range 10°C. to 0°C., the better the survival at each humidity, particularly at 50% RH. This did not agree with results obtained by Prasada (1948). He found that spores survived best at 5°C. to 7°C., and almost equally well at 10°C. to 15°C. as at 0°C. His uredospores became inviable after 22 weeks at 5°C. to 7°C. The difference between Prasada's results and these studies is probably not due to differences in the races used. Races differed only slightly under a wide range of storage conditions. The differences can probably be attributed to the germinating media used. It is assumed that Prasada used water or some other inert germinating media. The notoriously capricious germination of stored spores on such media, especially water, emphasizes the need for using host extract to assess viability.

In the normal process of spore metabolism the resting spore respires and uses up its respirable reserves. At the start these reserves appear to be greatly in excess of the minimum required to sustain maximum germination. During storage the level of the respirable reserves probably falls gradually to a certain critical level below which further deterioration may seriously affect the capacity of the spores to germinate. At this stage it is imperative that the spores germinate as rapidly as possible before the impact of high humidity and intimate contact with the germinating solution has so stimulated the respiration rate that the reserves fall below the level needed to sustain vigorous germination. Anything tending to delay germination would cause a serious deterioration in germination. Such a delay, among other things, could be caused by storage-hardening of the spores. This might be due to physical or physio-

logical deterioration of the spores tending to make them impermeable or dormant. Storage-hardening generally developed after several months of storage. It increased with age. But some samples became severely storage-hardened after only a few weeks of storage. The very capricious germination (after prolonged storage) of spores of race 1 kept at 50% RH at 0°C. and 3·5°C. suggested that this humidity induced a greater measure of storage-hardening than other humidities. But this may only have been due to aging of the spores which remained viable longer under these conditions than they did under other storage conditions.

Aqueous host extract countered the storage-hardening of the spores, stimulating the spores to germinate before serious deterioration of the respirable reserves had undermined the capacity of the spores to germinate. The physiologically active substance or substances were present in or comprised the oily fraction of the extract. The substances were readily extracted by boiling, but did not diffuse into solution when cold extracts were prepared by macerating tissue in a Waring blendor. The complex was not destroyed by prolonged boiling, but was destroyed by charring. It was ether-soluble and adsorbed by charcoal. The stag-horn development of the germ tubes at temperatures within the optimal range may have been a simple tactile response to the aqueous extract. It may, however, have been conditioned by the same substance or substances which stimulated germination. It should supply a simple means of determining temperature tolerance of different races.

Poor germination on gelatin and water was probably due to the fact that the preliminary period of conditioning of the spore contents leading up to actual emergence of the germ tubes was unduly prolonged by the storage-hardening of the spores. This permitted a considerable deterioration of respirable products. The better germination on gelatin could not be attributed to its physiological activity, but suggested rather that the physical spore-solution relationship played an important part in regulating the germination of storage-hardened spores. The rate of uptake of water by the spores may vary according to the germinating medium used.

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